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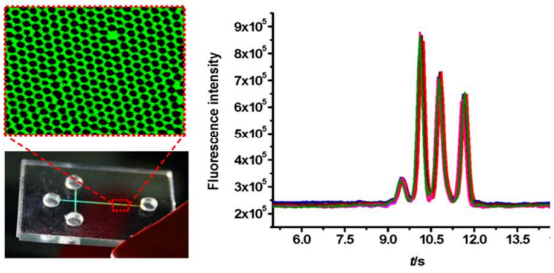

Highlight

We introduce an innovative one-step method to fast assemble and simultaneously hydrothermally immobilize PCs in a microfluidic channel.

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One-step packing of anti-voltage photonic crystals into microfluidic channels for ultra-fast separation of amino acids and peptides

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Abstract:

Packing of stable and crack-free photonic crystals (PCs) into micro channels is a prerequisite for ideal separation but often takes several days wasted in many steps including assembly and immobilization. This work was dedicated to finding a fast, one-step solution. Simply by heating and blowing away vapor, packing of silica PCs into micro channels by classic evaporation-induced assembly was greatly accelerated and could unite the immobilization into one step. An apt method was thus established, able to pack 2-cm PCs into microfluidic channels in 15 min, saving very much of the time. The packed PCs showed no evident cracks along the borders of their continuous domain, capable of anti-electrical field at 2000V/cm for 5h and storage in water for 2 months. This enables ultra-fast separation of amino acids along a 2.5-mm PC in 4s and peptides along a 10-mm PC in 12s. The separation was highly efficient and reproducible, with 300 nm plate height and 0.24%-0.35% relative standard deviation of migration time. This one-step approach is extendable to other gelling particles, and the resulted stable, crack-free PCs would have large potential in ultra-fast separation of other analytes.

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INTRODUCTION

Finding better separation materials has become critical in chromatographic researches and is far from completion.[1] Recently, photonic crystals (PCs) have been explored as a new type of theoretically idea media to differentiate the size and other properties of DNA,[2, 3] proteins[2, 4] and even small molecules.[5, 6] The so-called ideal PCs are meant continuously ordered structures which can be fabricated by either drilling holes through substances[7-9] or self-assembling the monodisperse particles on various supports.[10, 11] The assembling strategy is in practice useful and widely adopted for its simplicity, easiness in manipulation, high repeatability and low cost. The commonly used PCs are assembled from submicro or nano particles, possible to reach very high separation efficiency according to the chromatographic kinetic equation: [12]

$$H = 2\lambda d_p + \frac{2\gamma D}{u} + \mathrm{f(k)}\frac{{d_p}^2}{\mathrm{D}}u \qquad (1)$$

Where d_p is the diameter of packed particles, λ a factor decreasing with the improvement of packing uniformity until zero, γ an obstruction factor for diffusion in the packed bed depending also on the packing uniformity, D the molecular diffusion coefficient in mobile phase, $f(k)$ a function of the retention factor k, and u the mobile-phase linear velocity. Eqn. (1) shows clearly that the theoretical plate height H reduces or the chromatographic separation efficiency increases with the decrease of the packed particle size and the improvement of the packing uniformity. The first prediction has led to the wide use of very small packing in ultra-high pressure liquid chromatography (UPLC) ,[13-15] commonly 1-2 μm[16, 17] which are much smaller than those (3-30 μm) used in ordinary high performance liquid chromatography (HPLC). Unfortunately, the smaller are the particles packed, the higher becomes the backpressure (ΔP) because ΔP is inversely proportional to the square of particle diameter:

$$\Delta P = \phi\eta L u/{d_p}^2 \qquad (2)$$

Where ϕ is the flow resistance factor, η the solvent viscosity, and L the packed

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length. The UPLC thus keeps use of micro rather than nano particles in order to use mechanical pump(s).

The second prediction on packing uniformity is also a useful way to improve the separation efficiency but hard to achieve by a common packing method. It is due to the appearance of PCs that offer a new and practical way to have an insight into the influence of not only the particle size but also the packing uniformity on the separation. PCs also offer a new way to conduct nanofludic studies, which is at present a hot topic in addition to microfluidics.[18-20] By making full use of the orderly nano channel networks formed in PC bed and the large specific surface area on nano particles, ultra fast separation by PCs can be expected but not yet well explored.

Same as UPLC, PCs also suffer from the high backpressure, especially in case of nano PCs used. Mechanic pumps are not more the ideal means to drive mobile phase, instead, electro-osmotic flow (EOF) pump should be used. EOF is a bulk flat flow produced under direct electric field due to the existence of electric double layer on the particle surface and the inner wall of channels. It increases with the electric field so that the higher is the electric field, the faster will be the electric separation. The flat EOF is favorable for highly efficient separation because it can eliminate the parabolic-flow-caused broadening effect, but EOF and electric field can disband the orderly PCs if not properly immobilized.

Although Zeng *et al.*[2] found, in the separation of DNA, that PCs were stable at a low voltage (<200 V/cm) due to the existence of van de Waal's force and/or hydrogen bonds, immobilization of PCs is essential to withstand high voltage which is the key factor to improve the separation efficiency in electrochromatography. Several methods have since been reported. For example, Zheng *et al.*[6] fixed their PCs by sintering at 900^{o}C, which is a common way but easily creates cracks in PCs due to volume loss, affecting subsequent separations; Wei *et al.*[3] and Malkin *et al.*[21] immobilized silica PCs in capillaries by horizontal polymerization, reaching plate heights of ca. 50 nm and 100 nm for proteins and dyes, respectively; Park *et al.*[5] bonded PCs by pressing two plastic chips at 115^{o}C; And Sweedler *et al.*[22] integrated a

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nano-film into a three-layered chip, serving it as a frit to sustain the particles in microchannels. These methods are useful but take hours to several days. A faster method is thus expected if available.

In this paper, we suggest a one-step approach to fast prepare anti-voltage PCs. By heating and blowing away the vapor from one end of an assembling channel, the evaporation-induced assembly of PCs was greatly accelerated and successfully fused with the immobilizing step into one. The heat can accelerate both of vapor evaporation and silica particle gelling processes. The gelling could glue the particles together at quite a low temperature of 70°C. The mechanism is similar to the hydrothermal fixation of micro silica packing in capillary at a higher temperature.[23]

By this heat-accelerated one-step approach, crack-free and stable PCs (2 cm) were prepared with silica nanoparticles in 15 min. The resulted PCs could withstand an electrical field strength (*E*) up to 2000V/cm, able to separate amino acids in 4s and peptides in 12s. The column efficiency has reached 300 nm theoretical plate height measured from FITC, much better than the reported data.[5] These separations were highly reproducible, with the relative standard deviation (RSD) of migration time at 0.24% -0.35% and that of peak height at 1.1%-3.1%.

EXPERIMENTAL SECTION

Materials and Reagents

Tetraethylorthosilicate (TEOS, 98%) and ammonium hydroxide (NH3•H2O, 28%) were purchased from Alfa Aesar (Ward Hill, MA, USA). Fluoresceine-isothiocyanate isomer I was purchased from Acros Organics (Geel, Belgium). Oligoglycine (G) peptides (G_4, G_5 and G_6) were obtained from Sigma (St. Louis, MO, USA). Hydrogen peroxide (30%), nitric acid (65%) and sodium tetraborate (analytical reagent grade) were all obtained from Beijing Chemical Work (Beijing, China). Pure water used was from Milli-Q (Millipore, Milford, MA, USA).

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Fabrication of Microchips

Standard photolithographic and wet chemical etching techniques were used for fabricating channels onto a 1.7-mm thick glass plate with chromium and photoresist coating (Shaoguang Microelectronics Corp., Changsha, China). A photomask layout with two microchannels intersecting each other at right angles was transferred onto the glass plate through UV exposure. The exposed glass was etched in a mixture of concentrated HF-HNO_3-H_2O (20:14:66,v/v/v) to a depth of 30μm and width of 100μm.The injection channel (10 mm) was across the separation channel (20 mm) at their center points (10/2 and 20/2 mm from both their ends. Note: A single channel can also be used but the inlet has to be tapered and made hydrophobic to inject picoliters' sample into the PC channel). The reservoirs were drilled through the etched plate at the four ends of the channels with a diamond-tipped drill bit (0.5, 1.0, and/or 2.0 mm O.D.). A glass plate of 130μm thickness was used to cover the channels by permanent heat bonding. Briefly, a heavy corundum brick was placed on top of the two layered plates and heated, in a muffle furnace (Hua Gang Tong Tech. Co. Ltd., Beijing, China), at 10 ^{o}C/min to 100 ^{o}C (hold for 30 min), 10 ^{o}C/min to 550 ^{o}C (hold for 30 min), and 1 ^{o}C/min to 570 ^{o}C (hold for 4.3 h). The temperature was then lowered to 300^{o}C in 2 h, followed by natural cooling of the furnace to room temperature.

Derivatization of samples

Amino acids and peptides were derived with FITC using a procedure revised from literature.[24] Briefly, FITC (3 mg) was dissolved in 25 ml acetone to form a ca. 3.0310×10^{-4} M labeling solution and stored at -20 ^{o}C. The amino acids and peptides were individually dissolved in 5 mM borate buffer (pH 9.2) to form 10^{-3} M solutions. Each individual amino acid and peptide solution were mixed with the FITC labeling solution at a volume ratio of 20μl : 20 μl and kept in darkness at room temperature for overnight to complete the reaction. Before use, the labeled amino acids and peptides were diluted by 100–1000 times with running buffer. To form mixed samples, each

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individual FITC-amino acid and FITC-peptide were combined at the same volume ratio.

Synthesis of silica nano particles

Monodisperse silica microspheres were prepared by emulsifier-free emulsion polymerization according to the Stober-Fink-Bohn method.[25] Briefly, ethanol (73.8 ml), water (10.8 ml) and ammonia (9.8 ml, 28%) were first mixed, by magnetic stirring, in a round-bottomed flask warmed in a water bath at 25°C and TEOS (5.6 ml) was then quickly poured into the mixture. After a 12-h reaction, the particles were harvested by centrifugation and washed first with pure EtOH for 4 times and then with water until pH 7. The cleaned silica nano particles were sintered at 600 °C for 3 x 10h,[26] and re-dispersed in water at 10% (w/v) for long term storage. The prepared silica particles were all sphere as viewed by scanning electron microscopy (SEM) with a diameter of 500 nm.

Preparation of silica PCs

The process toprepare PCs in microfluidic channelsis schematically illustrated in Fig. 1. A glass chip (Fig. 1a) was first rinsed with 0.1M HNO_3 and water. Then 5-µl silica particle suspension, fully degassed by sonication for about 30 min, was injected into the reservoir 2. Once all the channels were automatically filled by the suspension due to capillary force, the remaining reservoirs 1, 3 and 4 were added with the same silica particle suspension and then evaporated by blowing a heated airflow at about 70°C against the reservoirs using a blow dryer while the reservoir 2 was continuously supplied with suspension. This assembled first a layer of ca. 100µm silica particles on the bottom of the three reservoirs, then silica PCs along the channel by drawing the suspension from the reservoir 2 (Fig. 1b). Once the assembled PCs reached the reservoir 2, the assembly was stopped by filling the reservoir 1, 3 and 4 with 5µl water each, and the reservoir 2 was blew with the same hot airflow until dryness (about 3min) to fix the end (Fig. 1c). The silica particles deposited in all the 4 reservoirs were then unstuck and renewed by water. The reservoirs should be kept

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filling with water to avoid drying out by natural evaporation (Fig. 1d).

Ultra fast separation on PCs

All PC-based separations were performed on a laboratory-built microchip system coupled with an inverted fluorescence microscope (IX71, Olympus, Japan) and four direct current high voltage power supplies (0–5000 V, from Dongwen High Voltage Power Supply Plant, Tianjing, China) connecting to the four reservoirs, respectively, by gold wire electrodes. The power supplies were independently controlled using laboratory-edited software via a multichannel digital / analogue convert board (model AC6302 from wwlab, Beijng, China). Before separation, the water in the reservoirs was all replaced by 5 mM $Na_2B_4O_7$ (pH 9.2) and equilibrated at 100V/cm. The reservoir 3 was then emptied, loaded with a sample solution and injected into the separation channel by electric clip injection (200V, 200V, 1300V and grounded for reservoir 1, 3, 2, and 4, respectively). Separation was typically performed at 1000 V/cm, with a "pull-back" voltage of 1500V applied to the reservoirs 3 and 4 to prevent the sample from leaking into the separation channel.

The separated bands were detected by collecting time-lapse intervals of fluorescence images. The fluorescence was excited at 470-495 nm and emitted at 520 nm. The images were collected through a 10× objective lens with 0.3 numerical aperture and detected using a single photon detector (ANDOR iXonDV885, Northern Ireland). In order to save computer space, the collection size of the images was reduced to a small region of 200×200 μm^2, which was able to completely cover most of channels adopted. Images were digitally collected at ~10-25Hz to suit for fast separations and analyzed with Andor SOLIS software (www.andor.com). The fluorescence intensity data were obtained by adding the intensity values stored in the pixels of selected regions (100μm across and 50 μm along the channel) on the sequential images to produce electrocharomatograms.

RESULTS AND DISCUSSION

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Heating assembly and fixation of PCs in microchannels

PCs are commonly prepared by a slow process of natural evaporation-based vertical assembly.[27-29] Inside a micro channel, it can take a few days to assemble a 3-cm PC column.[6, 21] If its immobilization is included, the time may be doubled. To speed up and simplify the fabrication, we combined the evaporation assembly and immobilization into one step and accelerated the process by heat. This has been achieved by just blowing a hot air stream across the reservoirs 1, 3 and 4 while keeping the reservoir 2 in particle suspension (Fig. 1b). The hot airflow heats up the suspension in the reservoirs, vaporizes and fast dries out the solvent in the reservoirs. The particles will then be deposited at the bottom of the reservoirs, aggrading the bottom for about 100 μm, which closes the channel outlets, stops the inside-channel particles moving out and also enlarges the evaporation surface to further speed up the assembly. The heat will unavoidably be transmitted to the head section of the inside-channel-assembled PCs through the dried particles and the chip material. This accelerates the assembled silica PCs to gel in water and glue each other. Thus fused silica particle network is formed and stabilized after cooling. It is designed to serve as a pseudo-frit to stop the movement of inner PCs.

It was found that the hot airflow had profound influence on the speed of PC formation. Under a same airflow, the assembly increased its speed with several factors such as evaporation area, airflow rate, and temperature, of which the temperature is the most critical and can easily be controlled. Raise of temperature can reduce the solvent viscosity and increase the saturated vapor pressure. The reduced viscosity helps to decrease the backpressure, which in turn speeds up the transportation of solvent to favor the evaporation. Such an increase is expected to gradually slow down because the solvent transportation resistance increases with the length of PCs in micro-channels. Fig. 2 shows that the assembling speed is an upward asymptote *vs.* temperature, rising from 0.97 to 2.1 mm/min as the temperature changes from 25 to 80^{o}C. The speed increases quickly at the low temperature phase and starts to slow down at ca. 60^{o}C. Although higher temperature can further increase the speed a bit, its

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increment is nearly negligible. The temperature between 70-80°C gives quite a similar assembling speed. A temperature higher than 80°C is thus not suggested to save energy and to prevent the PCs from cracking. By Fig. 2, it is clear that a temperature lower than 60°C could lose much the assembling speed and is also not adopted.

The hydrothermal stabilization[30] of PCs is also temperature-dependent. It can be expected that the higher is the temperature, the faster will be the dissolution of silica into the surrounding water to form polysilica acids at the outer surface of silica particles. High temperature is hence favored but somewhat different from the creation of silica frits in a capillary[23] which allowed to heat up to 110°C,[30] the PCs packed in microchannels could not be burnt or heated to a very high temperature otherwise, the PCs will be destroyed, making no way to pump solvent through the heated channel(s). Also different from capillaries, the microfluidic channels cannot be recovered by cutting off the channels piece by piece. To keep PCs in their assembled states, they should always be immersed in water which limits the heating to a temperature below 100°C. By taking into consideration of fast but reproducible assembly, the gelling temperature should further be lowered to below 80°C. Further investigation revealed that the PCs greatly lost their stability against the high voltage if they were immobilized at a temperature below 60°C (possibly much longer time needed to gel the particles). A temperature of 70°C was found to be able to compromise the assembling speed and immobilizing stability.

The same hydrothermal immobilization process also happens between the glass channel wall and the particles, which further strengthens the stability of the pseudo-frits no matter if there are particle-wall interactions or not.[26, 33]

Clearly, the temperature of 70°C required for thermal fixation of PCs is much lower than 115°C required to immobilize silica micro particles.[22] The probable reasons include use of nano-sized, unmodified silica particles (compared with the C18-columns). The uncoated silica particles are easier to dissolve in water than the C18-coated ones and the dissolution will be enhanced as the particle size decreases to enlarge the surface area for contacting more water. This will, in theory, contribute to

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reducing not only the fixing temperature but also fixing time.

By the selected temperature, silica PCs could be assembled at a speed of about 0.2 cm/min measured in a 30μm x 100μm channel filled with a suspension of 10% (w/v) 500 nm silica particles. The total time to pack 2cm of stable and crack-free PCs was about 15 min. Further studies demonstrated that this one-step method was somewhat universal, able to fabricate various stable and crack-free silica PCs with particle size ranging from 280 to 830nm, which was deduced from the data obtained with 280, 500, 710 and 830nm particles. The stability of the resulted PCs was checked by application of high electric field. They could all tolerate an *E* high up to 2000V/cm that is favorable for ultrafast separations. Their orderly structure maintained perfect even after a continuous application of 2000V/cm for 2 hours (Fig. 3d).

The orderly structure was characterized by confocal laser scanning microscopy [22, 31] rather than SEM which is destructive to PCs. By simply filling a FITC solution into the PC bed, the orderly structure at different channel section can be viewed clearly as shown in Fig. 3a and b. By this indirect method, the PC particles appeared as black objects while the FITC solution around the particles is green. Clearly the formed PCs have face-centered cubic cell with the (111) plane parallel to the substrate. The structure consists of several large perfect domains with continuous borders. It should be noted that there were randomly distributed or uneven points along the borders but they were too few to interrupt the continuity of the resulted PCs. It should also be noted that the fixed ends (Fig. 3c) were undistinguishable from the unfixed bed by this optical observation.

The reason responsible for the continuity of PCs is explained as follows: During assembly, the channels were always filled with particle suspension and kept to have an adequate supply of the suspension from the inlet reservoir. It is inside the microchannel that the solvent water cannot directly evaporate into the air but is forced to flow toward the channel ends or reservoirs blew by hot airflow. This enables a continuous flow of the water in the channel, bringing the suspended particles to the assembling sites. This mechanism eliminates the discontinuous assembly of PCs and

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avoids the formation of drying-induced cracks in the beds (Fig. 3d, Fig. S1a and b). This makes later control of modification and/or separation more easily and more exactly.

Format to stabilize PCs

In order to conduct fast and efficient electrochromagraphy at high electric field, the PCs packed in channels should be immobilized steadily otherwise, they can disband very easily at E>100 V/cm (Fig. S2a). We can have three ways to stabilize the assembled PC beds: fixing a section of PCs at their one or both ends or fixing the whole PC beds, all by the thermal gelatin approach. Fixing one end of the PCs is the simplest and showed to be capable of sustaining an E up to 1000 V/cm when the positive polar was applied opposite the fixed end (Fig. S2b). However, the PCs were disbanded at a low E as the voltage polar was reversed (Fig. S2c), much similar to the unfixed PCs (Fig. S2a). This one-end fixation will thus reduce the application range of PCs for 50% and is not suggested as a universal approach.

Instead, two-ends-fixation is proposed and demonstrated to work perfectly, independent of voltage polar applied. Fig. 3d validates the stability of the two-ends-fixed PCs (Tef-PCs), able to tolerate E up to 2000V/cm without any breakage. A bit higher E could be applied but not suggested for reproducible separation and long term use.

Total fixation[32] of the PCs was possible by heating the entire chip in hot water after all the ends of PCs were fixed by hot air. The totally fixed PCs have no problem to tolerate the electrical field strength of 2000 V/cm (Fig. S2d) or even higher (data not shown) to further facilitate the performance of ultra fast separations. But the total fixation complicates the fixing manipulation and has a risk to crack the well-arranged PC bed during heating and cooling due to expansion and shrinkage. It is hence not recommended.

Fig. 4a shows that the plot of current against time measured at 200V/cm. The Tef-PCs (Fig. 4a-A) gives the most constant current, demonstrating the maintenance

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of impedance or continuity of the PCs in the channel, but the one-end-fixed (Fig. 4a-B, with positive polar connecting to unfixed end) and not fixed (Fig. 4a-C) PCs give increased current, indicating the reduction of resistance or disbanding of PCs in the channels. Further experiments revealed that the Tef-PCs kept their expected current-time characteristic over a wide range of E high up to 2000V/cm (Fig. 4b), with good linearity between voltage and current (Fig. 4c) or obeying the Ohm's very well. The Tef-PCs could be continuously used for 5h or stably be stored by immersing in water for 2 months.

It is also attractive that the PC beds together with the fixed ends can be unpacked and evacuated by sonication and electromigration at 200 V/cm. After evacuation and cleaning of the channels**,** the chips can be re-assembled with PCs. This reduces preparing time and cost, and is able to make the PC-based separation more reproducible.

Performance of Tef-PCs

According to the good linearity of a wide range of E vs. current (Fig. 4c), high performance of the Tef-PCs were expected[21] and demonstrated by using FITC as an indicator and electrophoresed along a 2.2-mm Tef-PC under different electrical field strength. Fig. 5a shows that the peak is narrowed quickly as E increases, giving a good linearity of migration rate vs. E (Fig. 5b). The column efficiency is denoted by plate height H which was calculated by fitting the peak data into a Gaussian distribution to get standard deviation ρ in time and then by an equation of $H=\rho^2 L/t_R^2$, where L is the effective separation length and t_R the migration time. After the contribution of sample injection was subtracted,[33] the plate height was plotted against migration rate as shown in Fig. 5d where the smooth curve was plotted by fitting to the van Deemter equation.[34] Clearly, the column efficiency or H is E-dependent similar to literature.[4, 35] The plate height decreases very fast at the low migration rate and soon turns to a slow decline. The H starts to decline very slowly and nearly linearly at about 1.6 mm/s (corresponding to ~ 1000 V/cm). This point was considered

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as the starting point of high performance separation, able to produce H<300 nm for FITC, 30 times better than the reported data.[5]

In the case of PCs, the nano-scaled separation beds will make mass transfer very fast so that the C-term in the van Deemter equation is negligible. This is one reason for PCs to improve the separation efficiency. The second reason to increase the efficiency is the effective reduction of molecular diffusion by PC beds. By migrating a section of FITC into the packed channel and monitoring its peak variance *vs* time at E=0 as shown in Fig. 5c.[21] The slope of the B-term was obtained: $2\gamma D = 4.03\times 10^{-4}$mm^2/s where γD denotes effective molecular diffusion and it reduces to about 43% of the diffusion coefficient D of FITC in free solution,$4.41\sim4.61\times10^{-4}$mm^2/s.[36] The third and the most important reason to increase the efficiency is the effective reduction of eddy diffusion resulted from the small packing particles and the great elimination of heterogeneity of packing materials while using PC beds. This can be deduced directly from the A-term of the van Deemter equation which was obtained by nonlinear regression fitting with the experimental data, giving a value of 7.99×10^{-5}mm which is much smaller than common packed columns.[17, 34] Since the A- term is dependent not only on packing homogeneity but also on particle size, the further improvement of the column efficiency lies in the use of even smaller PC particles. By using 1200 and 740nm particles, we have measured the efficiency, giving 785 and 446 nm plate height, respectively when the migration rate is at about 1.6 mm/s. This clearly shows the direction for future studies.

Tef-PC-based separation of amino acids and peptides

To test the real applicability of Tef-PCs, four mixed amino acids were first chosen as a testing sample and separated at high electrical field strength. Fig. 6a demonstrates the fast (4s) separation of the amino acids through a section of only 2.5 mm PCs at room temperature, with H =0.5~2.3μm. The resolution and peak efficiency and running speed are much improved if compared with literature.[5] The largely

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improved separation speed and efficiency were first attributed to high *E* applied and hence fast electro-osmotic flow (EOF) produced, which is evidently due to the use of voltage-sustainable and crack-free Tef-PC medium. The voltage-sustainability allows the application of high electrical field strength while crack-free bed makes the sample bands migrate smoothly, avoiding extra broadening. The nano-sized hole network in PCs offers excellent mass transfer path ways and the tiny particles enlarge the stationary surface area for interaction with analytes. This is also an important mechanism responsible for the high resolution available in a very short (2.5 mm) PC bed.

Further validation of Tef-PCs was performed using peptides as the testing sample. By the bare-silica-particle-fabricated PCs, fast and efficient separation of oligo(Gly)s (G_4– G_6) was demonstrated as shown in Fig. 6b. In addition to the selection of perfect Tef-PCs, the pH value of running buffer was found to impact strongly on the separation as reported.[37] After some optimization, the peptides could be baseline separated, in 12s, through a 10mm PC bed at pH 10.02, with H=0.81~1.01μm. The separation speed is comparable with that of C18-modified silica colloidal stripe[5] but resolution and overall performance are clearly much improved. It should be mentioned that the Tef-PCs used has no C18 coating that can be fabricated more easily and more cheaply.

Reproducibility of Tef-PCs

The reproducibility of Tef-PCs was tested by using FITC and oligo(Gly)s as testing samples. Fig. S3a shows10 consecutive separations of FITC and peptides after the PC bed were used for 5h. The relative standard deviation (RSD) of migration time ranges 0.24% for G_6 to 0.35% for FITC while the RSD of peak height from 1.1% for G_4 to 3.1 % for G_5. Fig. S3b shows the case of using six different PC beds, the RSD of migration time ranges 3.4% for FITC to 7.2% for G_4 while that of peak height from 3.2% for G_6 to 6.8 % for G_4. These chip-to-chip and run-to-run data validated that the fast prepared stable PCs or Tef-PCs are in practice usable.

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CONCLUSIONS

In this work, we combined the evaporation-induced self-assembly of PCs and hydrothermal immobilization into one step to fast fabricate crack-free and durable PCs in microchip channels. This new one-step approach is simple, fast, low-cost and applicable to the packing of silica PCs in microchip channels with various immobilization formats. After comparison, high-voltage-sustainable Tef-PCs were proposed as a new type of stable PCs. They can be prepared very fast and sustain strong electrical field strength up to 2000v/cm, suitable for ultrafast and excellent separation of amino acids and some peptides, with *H* reaching sub-micrometer. This study demonstrates the great potential to fast fabricate perfect, stable PCs in microfluidic chips and to conduct ultra fast separation with a short but stable PCs.

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Acknowledgement

This research was financially supported by NSFC (Nos. 21027003, 21235007 and 91117010), MOST and CAS.

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CAPTIONS FOR FIGURES

Figures

Fig. 1 Flowchart to rapidly assemble stable PCs into a glass microchip channels (a) by heating with a hot air flow (b and c) to accelerate the evaporation-induced assembly (b) and to fix the assembled ends (b and c). The fixed PCs are ready for use after the particles in the reservoirs were emptied (d).

Fig. 2 Plot of assembling rate *vs*. evaporation temperature. The error bars represent standard deviations obtained from three replicate measurements.

Fig. 3 Confocal image of a silica-particle-assembled PC column taken from its center section (a) and the fixed end (b), together with a photograph of the fixed end (c), before (a, b and c) and 2 hours after (d) application of a strong electric field at 2000V/cm. To take the confocal images (a, b, and d), the PC channels were filled with 20 μM FITC solution, excited by a laser at 488 nm and measured at 520 nm. Bare silica particles (0.5 μm diameter) were used to assemble PCs.

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Fig. 4 Electric sustainability evaluated at a constant voltage of 200V/cm (a) and stepped voltage up to 2000 V/cm at an increment of 400 V/cm per hour (b and c), using Tef-PC column (a-A, b and c), one-end-fixed PC column (a-B, negative polar at fixed end) and unfixed PC column (a-C). The curves were plotted by current, I, *vs*. time, t, (a and b) or electric field, E, (c).

Fig. 5 Superimposed electropherogram (a) measured from 0.1 mM FITC (in a running buffer of 5mM $Na_2B_4O_7$ at pH 9.2) at an electric field of 200, 400, 800, 1200, 2000V/cm, respectively and detected after separation through 2.2-mm PC, giving the linear relationships of the migration rate of FITC *vs.* applied electric field (b), and the peak variance *vs*. time (c) but non-linear relationship of the plate height *vs.* migration rate (d) as expected, where the circles were the data from experimental measurements and the solid line is fitted, by nonlinear regression, via the van Deemter equation. To plot the curve (d), the diffusivity, D, was calculated from the slope, k, of the least-squares fitted curve (c) detected at E = 0V/cm: $\gamma D = k/2 = 2.01 \times 10^{-4}$ mm^2/s.

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Fig. 6 Application of PCs to the separation of FITC-labeled amino acids (20 μM each) at 1200 V/cm (a) and FITC-labeled peptides (10 μM each) at 1000V/cm (b) using a running buffer of 20mM $Na_2B_4O_7$ at pH 10.02.The efficient separation length was 2.5 and 10 mm, respectively. Other conditions are the same as Fig. 5.

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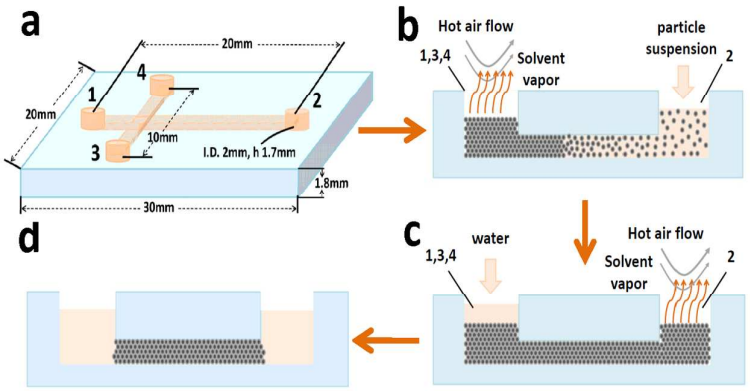
528 **Fig. 1**



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530 **Fig. 2**

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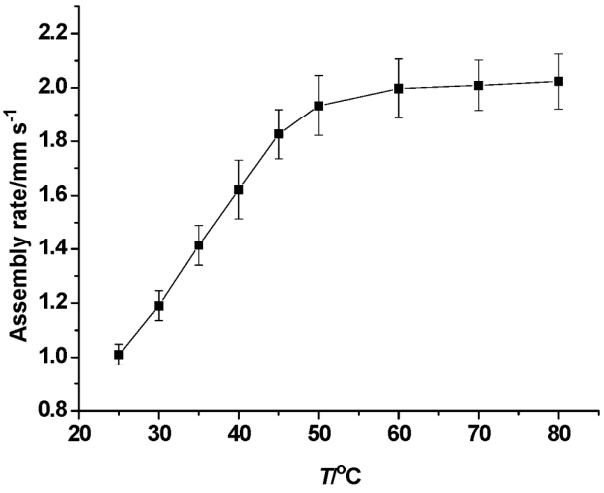

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536 **Fig. 3**

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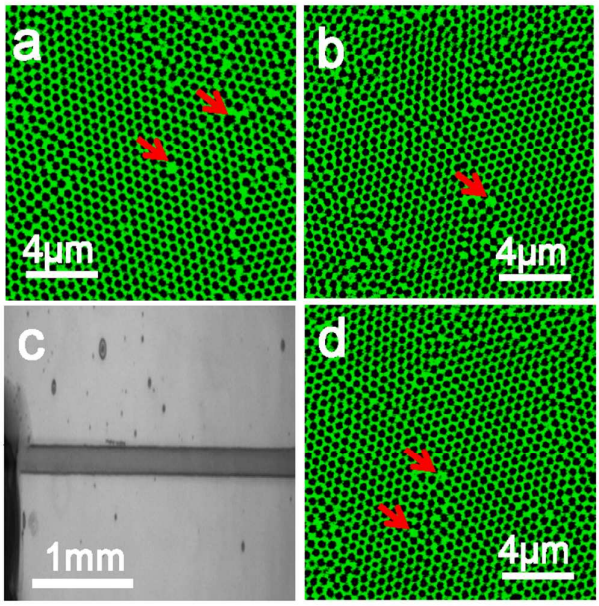
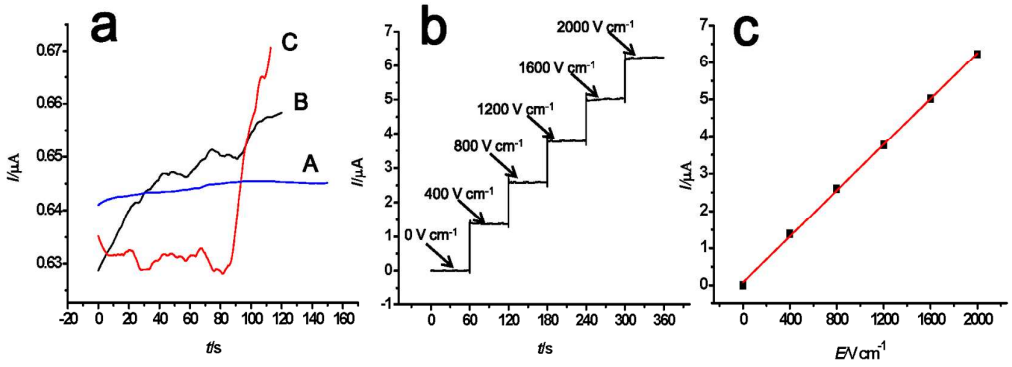


Fig. 4



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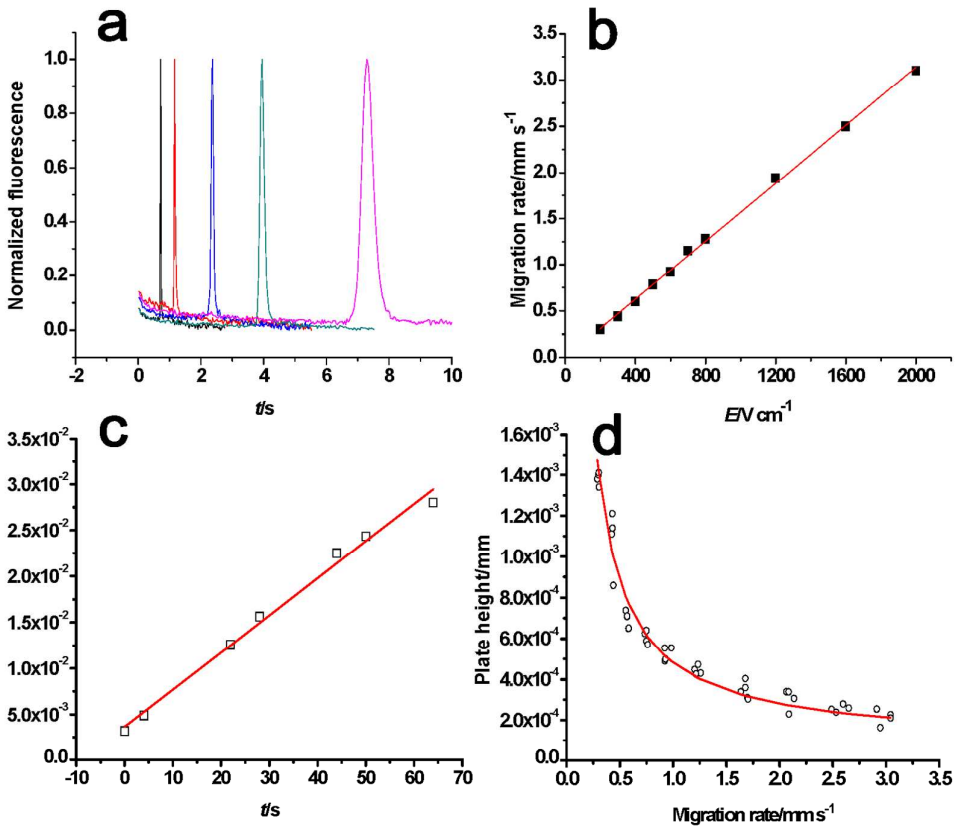
548 **Fig. 5**



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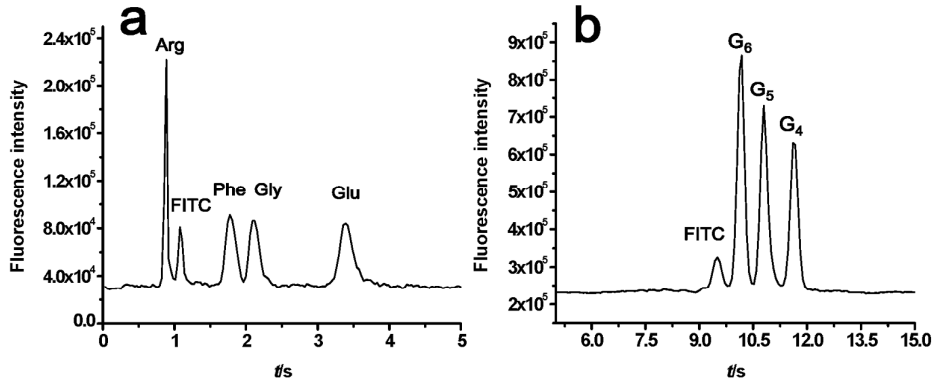
552 **Fig. 6**



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